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ORIGINAL PAPER

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The complete sequence of the human *CD79b* (Igβ/B29) gene: identification of a conserved exon/intron organization, immunoglobulin-like regulatory regions, and allelic polymorphism

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Abstract We determined the complete genomic sequence of the human *CD79b* (Igβ/B29) gene. The CD79b gene product is associated with the membrane immunoglobulin signaling complex which is composed of immunoglobulin (Ig) itself, associated in a noncovalent fashion with CD79b and a second polypeptide chain, CD79a (Igα/mb1). The sequence and exon/intron organization of the human and mouse *CD79b* genes are highly similar. The gene organization suggests that some variant forms of CD79b may arise by virtue of alternative splicing of mRNA. In addition, a number of conserved regulatory sequences commonly found in *Ig* genes are present in sequences which flank the human *CD79b* gene. Some of these sequences are distinct from those found in the *CD79a* promoter. These differences may explain why transcription of *CD79b*, but not *CD79a*, is observed in plasma cells. A new *Taq* 1 restriction fragment length polymorphism is described that is not associated with any structural polymorphisms of the expressed CD79b polypeptide.

The nucleotide sequence data reported in this paper have been submitted to the GenBank nucleotide sequence database and have been assigned the accession number L27587

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Introduction

The recognition of specific antigen by B lymphocytes depends on the function of the membrane immunoglobulin (mIg) receptor complex, a multimeric complex which, in addition to mIg, includes at least two associated glycoproteins designated CD79a (Igα/mb1) and CD79b (Igβ/B29) (Reth 1992; Schlossman et al. 1994). These immunoglobulin(Ig)-associated molecules participate in signal transduction and contain highly conserved intracytoplasmic domains which appear to associate with intracellular signalling molecules. The CD79b molecule is of particular interest because it appears that several different forms of CD79b may exist (Friedrich et al. 1993; Hashimoto and co-workers, unpublished results), the molecular basis of which remains undefined. In addition, unlike *CD79a* (Igα/mb1), the transcription of *CD79b* is maintained in mIg plasma cells (Hashimoto et al. 1993). In order to begin to understand these features of *CD79b*, we determined the complete sequence of the *CD79b* gene, and in the course of this work we also defined a new *Taq* I polymorphism.

Materials and methods

Isolation of cosmid clones

A chromosome 17-specific cosmid library was obtained from the Reference Library Data Base of the Imperial Cancer Research Fund (London, Great Britain). Filters #1 and #2 (Library number 105, set 12) were screened with a cDNA probe containing the full-length *CD79b* coding sequence, using standard conditions of high-stringency hybridization. Duplicate positive colonies were identified by position on the filter grid, and provided to us by the Reference Library.

DNA sequencing strategy

DNA sequencing was performed by using a *Taq* DyeDeoxy™ Terminator Cycle Sequencing Kit (ABI, Foster City, CA). Primers specific for portions of the *CD79b* gene were synthesized (Biosearch 8700; Millipore, Bedford, MA) as needed for sequence analysis by a primer walking strategy. Twenty-two primers were used to complete the se-

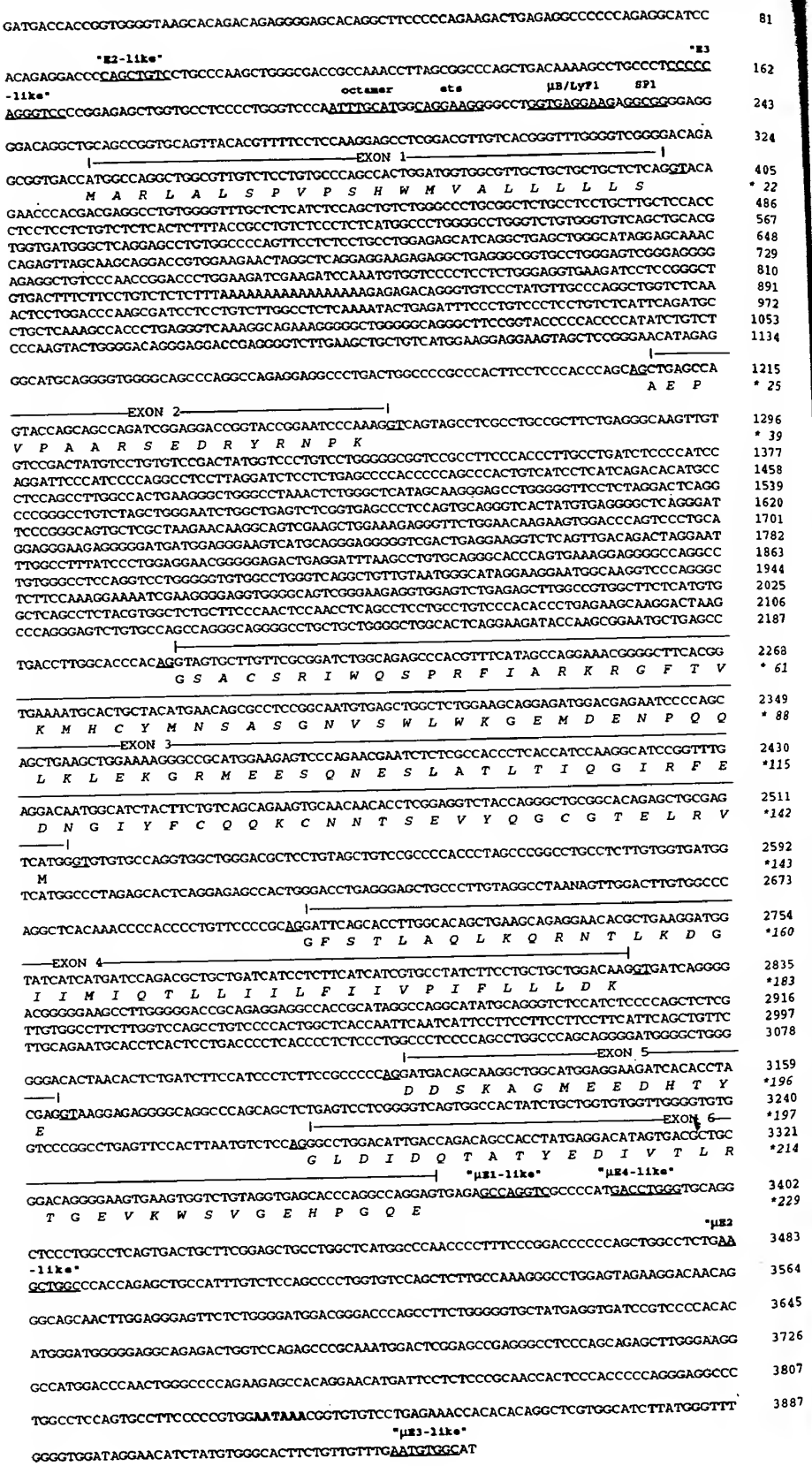

Fig. 1 The complete DNA sequence of the human *CD79b* (IgB/B29) gene. Potential regulatory sequences are *underlined* and labeled *above*. The exon/intron borders are indicated by a *horizontal line*, and the adjacent splice donor and acceptor sequences are *underlined*. The polyadenylation signal is indicated in *bold*

quence analysis of approximately 3800 base pairs (bp). Sequencing reactions were analyzed on an Applied Biosystems (Foster City, CA) Automated Sequenator Model 373A. The most 3′ sequencing primer F13 (5′ CGG ACA GGG GAA GTG AAG TG 3′) was used to confirm the 3′ extent of cosmid clones.

Southern blot analysis

Ten μg of genomic DNA were digested overnight with *Taq* 1 (Gibco/BRL, Gaithersberg, MD), electrophoresed through 1% agarose gel, and transferred onto nylon membranes (Micron Separations, Westboro, MA). After cross-linking using the Stratalinker UV cross-linker (Stratagene, La Jolla, CA) for 90 s, the filters were prehybridized for 15 min at 60° C in QuikHyb solution (Stratagene). Filters then were exposed for 1 h at 60° C in QuikHyb solution to a ^{32}P-labeled, 800 bp probe derived from the Igβ cDNA, and subsequently washed twice for 15 min at room temperature in 2 × standard sodium citrate (SSC), 0.1% sodium dodecyl sulfate (SDS), followed by a 30 min wash at 65° C in 0.1 × SSC, 0.1% SDS. Filters were exposed overnight on Kodak XAR5 film at –70° C.

Results

Isolation of cosmid clones for CD79b

The *CD79b* gene has been localized to chromosome 17q23 (Wood et al. 1993). We therefore obtained a chromosome 17-specific cosmid library for screening from the Reference Library Data Base of the Imperial Cancer Research Fund. The cosmid library was screened with a cDNA clone for human *CD79b* which was previously isolated in this laboratory (Hashimoto et al. 1993). Seven positive clones were identified on the initial screening. These clones were provided to us by the ICRF laboratories and we subsequently identified two cosmids which appeared to contain the entire *CD79b* gene. These clones were identified by their ability to be amplified by PCR primers specific for the 5′ end of the *CD79b* cDNA sequence (primer pairs F24/R5), and by successful priming of sequence reactions, using a primer (F13) specific for the 3′ end of the the cDNA sequence.

Sequence analysis of the CD79b gene

The entire genomic sequence of the *CD79b* gene was obtained and is shown in Figure 1. The overall intron/exon organization is highly similar to that reported for the mouse *CD79b* gene (Reth 1992). The *CD79b* genes of both human and mouse contain 6 exons. Exon 1 encodes a typical leader peptide sequence; this is followed by exon 2 which encodes a short peptide preceeding the Ig-like domain encoded by exon 3. Exon 4 encodes the hydrophobic transmembrane (TM) portion of the CD79b molecule, whereas the coding regions for the intracytoplasmic (CP) domains are divided between exons 5 and 6. As noted previously (Hashimoto et al. 1993), the mouse and human sequences are highly similar in sequence, especially in the TM and CP regions (>95% similarity in predicted amino acid sequence), as well as sharing the conserved exon/intron structure shown here. A 3′ untranslated (UT) region of 461 bp separates the stop codon from the polyadenylation signal AATAAA.

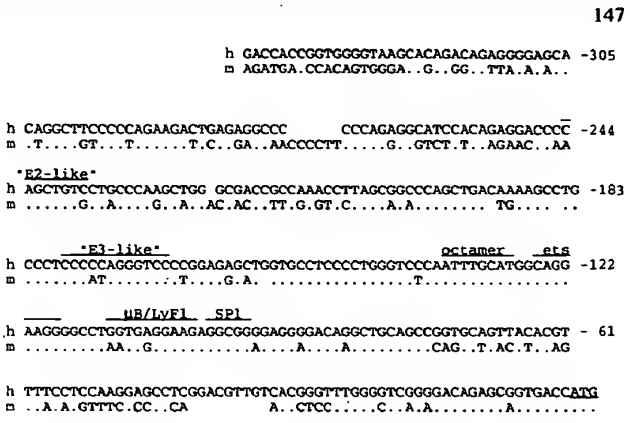

Fig. 2 Sequence comparison of the 5′ UT regions of the human and mouse (Hermanson et al. 1988) *CD79b* genes. Conserved regulatory sequences are indicated

In addition to interspecies similarities in the coding region and gene organization, there are also conserved *cis*-acting regulatory elements which are present in the flanking sequence of human *CD79b*. A comparison of the human and mouse 5′ promoter region is shown in Figure 2. This region contains sequences which are known to be involved in the regulation of *Ig* genes. Among these are the highly conserved octomer sequence, as well as an ets binding site and an SP1 target sequence. A uB/LyF1 binding region was identified in the mouse and is partially conserved in the human sequence. An additional enhancer (E3-like) element was tentatively identified in the previously reported mouse sequence, and is present in a slightly altered form in the human *CD79b* promoter. An E2-like sequence is also found in the human sequence, but was not observed in the mouse (Hermanson et al. 1989). Both the human and mouse promoter sequences lack a 5′ TATA box. Consistent with this, the mouse and human *CD79b* transcripts have been shown to initiate at multiple sites downstream of the octomer sequence (Hermanson et al. 1989; Omori and Wall 1993).

Enhancer elements can also be found in the 3′ end of *Ig* genes (Staudt and Lenardo 1991), and for this reason we searched the 3′ region of human *CD79b* for such sequences. We identified four potential sites of E elements, all of which had 7/8 nucleotides identical to the previously described E elements (Staudt and Lenardo 1991). These are indicated in Figure 1.

Identification of RFLPs in the human CD79b gene

We identified an allelic polymorphism associated with the *CD79b* gene after digestion with *Taq* 1. As shown in Figure 3, two bands are observed in homozygous individuals. One band at 2.2 kilobases (kb) is constant in all individuals. A second band reflects a *Taq* 1 polymorphism which is biallelic in the population, with either a 1.8 kb or 2.5 kb

Fig. 3 Southern blot analysis of *Taq* I-digested genomic DNA from five unrelated individuals. A full-length *CD79b* cDNA probe was used. Two alleles have been observed in the population. Lane 1 shows an individual homozygous for the 2.5 kb allele, while lanes 2–4 show individuals homozygous for the 1.8 kb allele. The individual in lane 5 is heterozygous. All subjects contain a constant, and relatively weak, band at 2.2 kb

Table 1 Distribution of Igβ *Taq* I RFLPs in various ethnic groups.

	Caucasian (n = 20)	Black (n = 20)	Oriental (n = 20)	Total (n = 60)
2.5/2.5*	3	1	4	8
2.5/1.8	8	3	9	20
1.8/1.8	9	16	7	32

An asterisk indicates that Southern blot analysis of *Taq* I-digested genomic DNA, using a full-length *CD79b* cDNA probe, identified two polymorphic fragments of 2.5 kb and 1.8 kb

band present. Heterozygotes (lane 5, Figure 3) contain both of these bands, as well as the constant 2.2 kb band.

We analyzed 60 unrelated individuals for the presence of the *Taq* I RFLP. Subjects from various ethnic groups were examined, and Table 1 shows the results of this study broken down by ethnic group. All three ethnic populations appear to be in Hardy Weinberg equilibrium (null hypothesis not rejected by goodness of fit test).

Discussion

In this study we determined the complete sequence of the human *CD79b* (Igβ/B29) gene, and the sequence reveals a remarkable degree of similarity between the mouse and human homologues. These similarities relate to both sequence conservation within the coding region, as well as preservation of the overall exon/intron organization. The gene organization is of some interest for understanding the mechanisms by which variant forms of the CD79b molecule may be produced. It has recently been reported that a truncated form of mouse CD79b protein exists in which the C terminal portion of the cytoplasmic tail is deleted (Friedrich et al. 1993). The exact extent of this deletion has not been determined, nor has this been reported as yet in human CD79b.

Nevertheless, the exon/intron structure of the gene suggests that a possible mechanism for this deletion might be alternative splicing at the 3′ end of the gene, such that exon 6 is removed from some transcripts. In addition, using an RNAse protection assay, we observed truncated transcripts of the human *CD79b* in which a large segment of the extracellular portion of the molecule had been deleted (unpublished results). Interestingly, this deletion corresponds exactly to the removal of exon 3, again suggesting alternative splicing as a mechanism for this variant transcript. The functional significance of these variant forms of *CD79b* has not yet been established.

A second interesting feature of the Ig-associated signaling molecules is their distinct pattern of transcriptional regulation. *The CD79b* gene is transcriptionally active throughout all stages of B-cell differentiation (Hermanson et al. 1989). Thus, *CD79b* is expressed with approximately the same timing as the onset of Ig heavy chain rearrangement, an event associated with *Ig* gene transcription (Blackwell et al. 1986). Consistent with this pattern of expression, the *CD79b* gene contains regulatory elements commonly found in Ig enhancers. Some of these elements, such as the octomer motif, are identical in the mouse and human *CD79b* sequences (see Figure 2), as well as in the *Ig* genes (Staudt and Lenardo 1991). This sequence is known to form a binding site for transcription factors Oct1 and Oct2. Other putative enhancer elements also exhibit similarity to the previously defined Ig enhancer sequences. Among these are the E3-like and E2-like sequences present in the human *CD79b* promoter region. It has recently been shown that the E3-like sequence in mouse Igβ binds nuclear extracts in gel shift assays (Omori and Wall 1993). In addition to these 5′ enhancer elements, we have also identified candidate enhancer sequences in the 3′ end of the gene, similar to the location of such enhancers in *Ig* genes. Four such regions are shown in Figure 1, each of which matches the published Ig enhancer elements in 7/8 nucleotides. Further studies will be required to determine whether these putative regulatory motifs are actually involved in the regulation of *CD79b* transcription.

As described previously, the major function of the CD79b gene product is in the formation of a membrane signalling complex associated with Ig at the surface of B cells (Reth 1992). At a minimum, this complex consists of Ig itself associated in a noncovalent fashion with *CD79b* and a second polypeptide chain, CD79a (Igα/mb1). As we have reported previously, the *CD79b* gene is transcribed at high levels in plasma cells (Hashimoto et al. 1993). In contrast, the *CD79a* gene is not transcribed in plasma cells (Ha et al. 1992), consistent with the absence of surface Ig in these cells. The *CD79a* gene contains a promoter region which bears partial similarity to that seen in *CD79b*; both the ets and SP1 binding sites are present in the *CD79a* promoter, which also lacks a TATA box. However, no E3-like element is present in *CD79a*. In addition, a putative *cis*-acting sequence has been recently identified in *CD79a* which is the target for a novel DNA binding protein, BlyF (Feldhaus et al. 1992). BLyF is not present in plasma cells, and this may partially account for the lack of transcription of *CD79a* in plasma cells. Interestingly, a BlyF binding motif is not present in the *CD79b* 5′ promoter region, again emphasizing the distinct patterns of regulation for these two genes. Given the absence of membrane Ig in plasma cells, it is at present unclear what function *CD79b* has at this stage of B-cell differentiation.

Wood and co-workers (1993) have recently shown that the human *CD79b* gene maps specifically to 17q23. This is of interest in view of the fact that translocations involving

17q23 have been observed in some cases of lymphocytic leukemia (Mitelman et al. 1990). It is tempting to speculate that the presence of numerous enhancer elements for B-cell transcription within the *CD79b* gene might enhance the expression of oncogenes translocated into this region. However, to our knowledge, a detailed molecular analysis of these translocations has not been performed.

Finally, we identified a new *Taq* 1 polymorphism of the *CD79b* gene. As shown in Table 1, a preliminary analysis of gene frequencies within various ethnic groups shows a relative preponderance of the 1.8 kb *Taq* 1 allele with an overall heterozygosity (H) of 0.33; the distribution of genotypes is consistent with Hardy-Weinberg equilibrium in all three ethnic groups. We did not detect any coding region polymorphisms associated with this *Taq* 1 RFLP, although we have not yet searched for other allelic polymorphisms in the flanking regulatory regions.

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